Remarks

New claims

Claims 1 and 22 have been amended. Claims 41-44 have been added. These claims read on the elected species. These claims are fully supported in the specification as originally filed, as shown below. Thus no new matter has been added by these amendments.

Claims	Specification
1. (Currently amended) A method to promote wound healing in a patient, comprising: administering a nucleic acid encoding a growth factor operably linked to a promoter to wounded tissue of a patient at a wound site; and applying an electric field intradermally to the wounded tissue at the wound site in an amount sufficient to increase transfection ~~expression~~ of the nucleic acid encoding the ~~encoded~~ growth factor.	[26] In one embodiment of the invention, the gene delivery vehicle comprises **a promoter and a growth factor coding sequence**...Other promoters which can be used include the Moloney virus LTR, the CMV promoter, and the mouse albumin promoter. [51] 50 μL of the appropriate concentration of Luciferase plasmid was injected **intradermally** both anterior and posterior to each wound. In diabetic animals 10 μg of appropriate plasmid in 50 μL of media was injected anteriorly as well as posteriorly in the wound edges on both sides. The resulting skin blebs confirmed intradermal delivery of the plasmid and were marked with indelible ink. Wounds were left undressed and animals were housed individually. [52] Animals were **electroporated at the site of injection** within two minutes of plasmid administration, using a square wave electroporator (ECM 830, BTX Genetronics, San Diego, CA). A custom designed pin electrode, consisting of two 10 mm rows of parallel needles separated by 5 mm was used to apply the electroporation voltage (Figure 8).

	[59] We demonstrate that electroporation is a simple, safe, and efficacious means of **improving transfection** efficiency in skin wounds. The application of high voltage, short duration, square wave electrical field pulses **to wounded tissue** can enhance gene expression over 10 fold.
22. (Currently amended) A method to promote wound healing in a patient, comprising: administering a nucleic acid encoding a HIF 1-α operably linked to a promoter to wounded tissue of a patient at a wound site; and applying between 1 and 20 pulses of between 500 and 2,000 V/cm and between 10 and 1000 microseconds intradermally to the wounded tissue at the wound site, whereby wound healing is stimulated.	[26] In one embodiment of the invention, the gene delivery vehicle comprises **a promoter and a growth factor coding sequence**...Other promoters which can be used include the Moloney virus LTR, the CMV promoter, and the mouse albumin promoter. [51] 50 μL of the appropriate concentration of Luciferase plasmid was injected **intradermally** both anterior and posterior to each wound. In diabetic animals 10 μg of appropriate plasmid in 50 μL of media was injected anteriorly as well as posteriorly in the wound edges on both sides. The resulting skin blebs confirmed intradermal delivery of the plasmid and were marked with indelible ink. Wounds were left undressed and animals were housed individually. [52] Animals were **electroporated at the site of injection** within two minutes of plasmid administration, using a square wave electroporator (ECM 830, BTX Genetronics, San Diego, CA). A custom designed pin electrode, consisting of two 10 mm rows of parallel needles separated by 5 mm was used to apply the electroporation voltage (Figure 8).

	[59] We demonstrate that electroporation is a simple, safe, and efficacious means of **improving transfection** efficiency in skin wounds. The application of high voltage, short duration, square wave electrical field pulses **to wounded tissue** can enhance gene expression over 10 fold.
41. (New) The method of claim 1 wherein pin electrodes are used to apply the electric field.	[52] Animals were electroporated at the site of injection within two minutes of plasmid administration, using a square wave electroporator (ECM 830, BTX Genetronics, San Diego, CA). A custom designed **pin electrode,** consisting of two 10 mm rows of parallel needles separated by 5 mm was used to apply the electroporation voltage (Figure 8). Between 6 to 18 square wave pulses were administered, at an amplitude of between 400and 1800 volts, a duration of between 100 μs to 20 ms, and an interval between pulses of 125 ms (Figure 9).
42. (New) The method of claim 1 wherein the wounded tissue is at the wound's border.	[39] We assessed the ability of in vivo electroporation to enhance gene expression. Full thickness cutaneous excisional wounds were created on the dorsum of female mice. A Luciferase encoding plasmid driven by a CMV promoter was injected at the wound border. Following plasmid administration, electroporative pulses were applied to injection sites. Pulse parameters were varied over a range of voltage, duration, and number. Animals were sacrificed at intervals after transfection and the Luciferase activity measured. Application of electric pulses consistently increased Luciferase expression. The electroporative effect was most marked at a plasmid dose of 50 μg, where an approximate 10-fold increase was seen. Six 100 μs duration pulses of 1750 V/cm were found to be the most effective in increasing Luciferase activity. High

	numbers of pulses tended to be less effective than smaller numbers. This optimal electroporation regimen had no detrimental effect on wound healing. Electroporation increases the efficiency of trans gene expression and may have a role in gene therapy to enhance wound healing. A HIF 1-alpha encoding plasmid driven by a CMV promoter was then injected at the **wound borders** of homozygous diabetic mice and found to accelerate wound healing. The enhanced healing was more pronounced in electroporated animals.
43. (New) The method of claim 1 wherein pin electrodes are used to apply the electric field to the wound's edges.	[51] Female 6-8 week old BALB-c and BKS.Cg-m Lepr$^{db/db}$ (homozygous diabetic) mice were obtained from Jackson Laboratories (Bar Harbor, ME). All procedures were approved by the Johns Hopkins University Animal Care and Use Committee. Animals were anesthetized with an intraperitoneal injection of 0.02 ml/g of a 1.25% Avertin solution. Their dorsum was shaved and two symmetrical full thickness excisional wounds were created on their backs on both left and. right sides using a 5 mm punch biopsy instrument or in the case of diabetic mice with a 4 mm punch biopsy instrument. 50 μL of the appropriate concentration of Luciferase plasmid was injected intradermally both anterior and posterior to each wound. In diabetic animals 10 μg of appropriate plasmid in 50 μL of media was injected anteriorly as well as posteriorly in the **wound edges** on both sides. The resulting skin blebs confirmed intradermal delivery of the plasmid and were marked with indelible ink. Wounds were left undressed and animals were housed individually. [52] Animals were electroporated at the site of injection within two minutes of plasmid administration, using a square wave electroporator (ECM 830, BTX Genetronics, San Diego, CA). A custom designed **pin electrode**, consisting of two 10 mm rows of parallel needles separated by 5 mm was used to apply

	the electroporation voltage (Figure 8). Between 6 to 18 square wave pulses were administered, at an amplitude of between 400and 1800 volts, a duration of between 100 μs to 20 ms, and an interval between pulses of 125 ms (Figure 9).
44. (New) The method of claim 1 wherein the nucleic acid is a plasmid and the step of administering employs intradermal injection.	[51] Female 6-8 week old BALB-c and BKS.Cg-m Lepr$^{db/db}$ (homozygous diabetic) mice were obtained from Jackson Laboratories (Bar Harbor, ME). All procedures were approved by the Johns Hopkins University Animal Care and Use Committee. Animals were anesthetized with an intraperitoneal injection of 0.02 ml/g of a 1.25% Avertin solution. Their dorsum was shaved and two symmetrical full thickness excisional wounds were created on their backs on both left and. right sides using a 5 mm punch biopsy instrument or in the case of diabetic mice with a 4 mm punch biopsy instrument. 50 μL of the appropriate concentration of Luciferase **plasmid was injected intradermally** both anterior and posterior to each wound. In diabetic animals 10 μg of appropriate plasmid in 50 μL of media was injected anteriorly as well as posteriorly in the wound edges on both sides. The resulting skin blebs confirmed **intradermal delivery of the plasmid** and were marked with indelible ink. Wounds were left undressed and animals were housed individually.

Information Disclosure Statement

Applicant resubmits a patent reference, Lennox, US 6280411, which was not previously considered by the examiner. No explanation for the non-consideration was provided.

Claim objections

Claim 24 has been amended to supply the missing punctuation at the end of the claim.

Rejection of Claims 1-7, 14, 15, and 17-24 under 35 U.S.C. §112. first paragraph

Claims 1-7, 14, 15, and 17-24 stand rejected as not enabled for the full scope of the claims. The PTO asserts that the claims are broader than the enablement in that they do not specify that the nucleic

acid encoding the growth factor is operably linked to a promoter. Moreover, the PTO asserts that the expression of an electric field "sufficient to increase expression" is misleading because the electric field increases transfection, and does not directly increase expression. Applicants have amended the claims to address these two issues. The claims now recite linkage to a promoter and recite that the electric field is sufficient to increase transfection. Withdrawal of this amendment is respectfully requested.

Rejection of Claims 1, 2, 3, 5, 6, 7, 19, and 21 under 35 U.S.C. § 103(a)

Claims 1, 2, 3, 5, 6, 7, 19, and 21 stand rejected as unpatentable over a single reference, Zhang et al., U.S. 6,972,013. This rejection is respectfully traversed.

To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. M.P.E.P. §2143. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The present rejection fails on all three criteria.

The PTO cites Zhang as teaching ***non-invasive*** in vivo electroporation to skin. In contrast, the present invention utilizes ***invasive*** in vivo electroporation through the skin. Specifically the present invention utilizes intradermal application of an electric field. Moreover, the present invention utilizes administration of nucleic acid and electric field to wound tissue, which Zhang neither teaches nor demonstrates. Zhang would not have motivated one of skill in the art to use invasive electroporation; Zhang teaches only non-invasive electroporation. Zhang would not have suggested to one of skill in the art that administration of nucleic acid and an electric field directly to wound tissue rather than healthy skin would be successful. The success of the present method was not predictable based on Zhang's teachings.

Withdrawal of the present invention is respectfully requested as Zhang does not teach all elements of the claims, Zhang does not suggest modifying his teaching to make the claimed invention, and even if Zhang had taught all elements of the claims, one of ordinary skill in the art would not have had a reasonable expectation of success.

Rejection of Claims 1-7, 15, and17- 21 under 35 U.S.C. § 103(a)

Claims 1-7, 15, and17- 21 stand rejected as unpatentable over Bureau (WO 99/01157, English equivalent is US 6528315) taken with Ruben (20030186904). This rejection is respectfully traversed.

Bureau is cited as teaching "electroporation to deliver transgenes to cells of the tissue being treated for either dysfunction of the cells themselves or regeneration of vascularization by angiogenic

factors produced by the transgene." Office Action at page 11. This is fundamentally different than the present invention as claimed. The present invention does not treat a cellular dysfunction but rather treats wounds. Bureau does not teach or suggest treating wounds.

Ruben does not teach any in vivo treatment at all. Ruben teaches the electroporation of bacterial cells in culture to produce growth factors in culture. There is no suggestion that the growth factors could be made in situ in an animal by transfection and electroporation rather than in culture.

There is nothing in the prior art cited which indicates that one should combine the teachings of the two references. There is nothing in the prior art which indicates that if one did combine the teachings, the combination should have all the elements of the claimed invention, namely administration of nucleic acid to wound tissue and intradermal administration of an electric field to wound tissue. As already stated above with respect to Zhang, even if such a combination was suggested, one of skill in the art would not have been able to predict that the combination would be successful. Thus the rejection fails to make a *prima facie* case because it fails to teach all elements of the claims, there was no motivation to combine the teachings of the two references, and because there would have been no reasonable expectation of success, even if such a combination were suggested.

Because the rejection fails to make a *prima facie* case, withdrawal is respectfully requested.

Rejection of Claims 1, 14, and 22-24 under 35 U.S.C. § 103(a)

Claims 1, 14, and 22-24 stand rejected as unpatentable over Bureau (WO 99/01157, English equivalent is US 6528315) taken with Ruben (20030186904) and further in view of Arbeit (US 6838430). This rejection is respectfully traversed.

As discussed above, Bureau and Ruben fail to make a *prima facie* case. Arbeit is cited as teaching a nucleic acid encoding HIF-1α to accelerate wound healing in a patient. Arbeit teaches administration of variant nucleic acid sequences using viral vectors, liposome formulations, direct transfection, a gene gun, and ex vivo transfection followed by autologous cell transfer. Arbeit, however, does not cure the underlying failures of the primary references. The primary references do not teach intradermal administration of an electric field to wound tissue. Arbeit does not remedy this defect. The primary references do not teach or suggest the modification or combination of their teachings to form the presently claimed method. Arbeit does not remedy this defect. The primary references do not provide a reasonable expectation of success that intradermal administration of an electric field to wound tissue

would increase transfection and/or wound healing. Arbeit does not remedy this defect. Thus the three-reference rejection also fails to provide a *prima facie* case of obviousness. Withdrawal of this rejection is respectfully requested.

Respectfully submitted,

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